Direct Observation of Biomimetic Mineral Nucleation by Surface X-ray Scattering
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Introduction: Two aspects of the surrounding environment may be important for the control of biogenic and biomimetic mineral nucleation: the chemical species present, which can affect the rate of mineralization, and a substrate material or template which can stabilize various crystal polytypes or morphologies. A model system consisting of a liquid subphase and a surfactant monolayer has attracted particular attention, since the monolayer template's surface charge and lattice spacing may be tuned through careful choice of the organic surfactant, and by the application of surface pressure [1]. Similarly the ingredients of the subphase can be controlled, and may include the nucleating species along with additional metals or organic molecules thought to affect the kinetics of mineralization. Studies up to now have relied mainly on optical microscopy and ex-situ techniques like electron microscopy and electron diffraction — no *in-situ* probe on atomic length scales has been available.

Methods and Materials: We have used *in-situ* x-ray reflectivity and grazing incidence diffraction to directly observe the growth of an amorphous mineral precursor phase [2] at the arachidic acid–water interface. The supersaturated calcium carbonate subphase was prepared by dissolving $CaCO_3$ powder in water with bubbled CO_2 gas, with the addition of 25µg/ml poly(acrylic acid, sodium salt) (MW 8000) to slow the rate of nucleation and prevent the growth of macroscopic calcite crystals [3]. An arachidic acid monolayer was spread from chloroform solution and compressed to a surface pressure of 25 mN/m, after which the film was allowed to relax at fixed area. X-ray reflectivity and grazing-incident in-plane diffraction were monitored over the course of the next 20 hours to observe growth of the mineral film.

Results: The first reflectivity scans (Figure 1(a), top curves) show oscillations corresponding to the constructive interference from the monolayer–air and monolayer–water interfaces. As time progresses, a sharp peak at low q develops, indicating that a dense, thick film is growing at the monolayer–water interface. Fits to the reflectivity curves (Figure 1(a), lines) allow us to extract model density profiles, shown in Figure 1(b), inset. The monolayer alone (Figure 1(b) inset, red short-dash line) shows features corresponding to the monolayer headgroup and hydrocarbon tails, including a Stern layer of calcium ions within the headgroup plane. Model fits at later times indicate the dense film underneath the monolayer (solid and long-dash lines). The mineral film grows to a maximum thickness of 280 Å, at a rate of about 20 Å/hour. The density of the film is constant, with an electron density of 1.4 times that of water. In-plane diffraction from the arachidic acid monolayer remained unchanged during this 20 hour interval, with no other peaks appearing at prominent calcite or vaterite positions. However, after 20 hours had elapsed, weight on in-plane peaks began to shift to other q positions. Further studies on the crystallization phase of this system are in progress.

Conclusions: We have presented the first *in-situ* observations of biomimetic calcium carbonate growth, from its inception as calcium ions collected at a monolayer interface, to a macroscopic amorphous mineral film. The density of the film is 82% that of calcium carbonate hexahydrate, and about 50–60% that of dehydrated calcium carbonate phases. While incomplete coverage of a dense phase is possible, it is more likely that the mineral precursor is a highly hydrated calcium carbonate phase. Our further measurements have shown that in this system, the polymer concentration and molecular weight drastically affects the film growth, while we have no strong evidence for interactions between the in-plane monolayer structure and that of the mineral film. Hence, we believe that this system is more properly described as kinetically driven, rather than template driven. Control of biomimetic mineral films by a more direct effect of the surfactant template is presumably more important in other systems, which we hope to identify in the future.

References:
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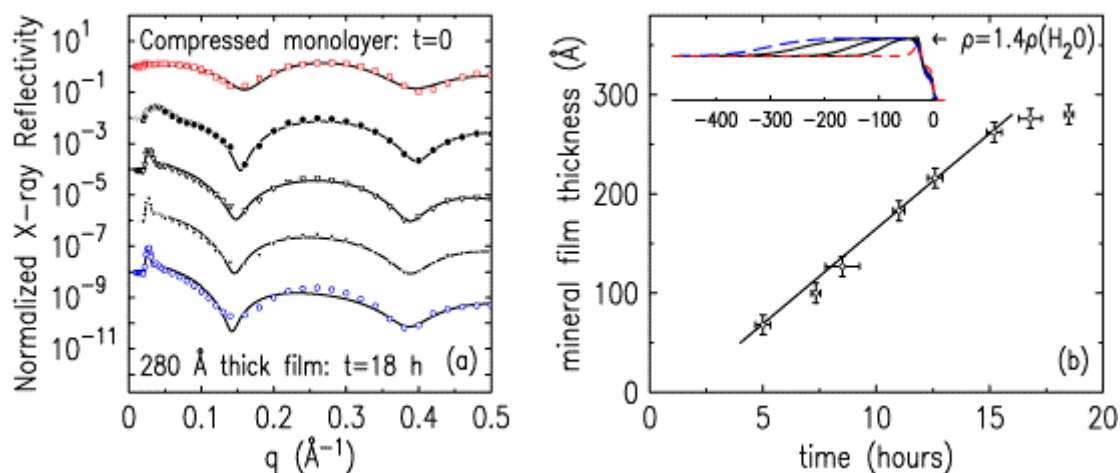[4] L. G. Parratt, Physical Review **95** (1954) 359.

Figure 1. (a) Fresnel-normalized reflectivity from compressed arachidic acid monolayer on a supersaturated calcium carbonate / polyacrylic acid subphase, beneath which an amorphous calcium carbonate film is developing over the course of about 18 hours. Lines are calculated from fit model density profiles, using the Parratt formalism [4] for $q < 0.1$ Å^{-1} and the Born approximation at higher q. (b) Thickness of the mineral film vs time. The line is a guide for the eye and indicates a rate of 20 Å /hour. Inset: selected model density profiles fit to the data, with the horizontal axis scale in Å. (- - -): initial condition, with arachidic acid headgroups / Ca^{2+} ions and hydrocarbon tails sticking up from the surface. (—): films of intermediate thickness. (– –): model of thickest observed film. Beyond this point, the film began to crystallize.